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PROVISIONAL APPLICATION FOR PATENT COVER SHEET

This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR §1.53(c).

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TITLE OF THE INVENTION (500 characters max)

Process

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ENCLOSED APPLICATION PARTS *(check all that apply)*

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[] Applicant Claims small entity status. See 37 CFR 1.27.

[X] A check or money order is enclosed to cover the filing fees.

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The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government.

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Respectfully submitted,

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PROCESS

The present invention relates to a process for introducing an antimicrobial material into a foodstuff. The present invention further relates to an antimicrobial material.

Background

Bacteriocins are antimicrobial proteins or peptides that can be produced by certain bacteria, which can kill or inhibit the growth of closely related bacteria. The bacteriocins produced by lactic acid bacteria are of particular importance since they have great potential for the preservation of food and for the control of foodborne pathogens. (Wessels et al. 1998.)

The most well known bacteriocin is nisin, which is the only bacteriocin currently authorised as a food additive. Nisin is produced by fermentation of the dairy starter culture bacterium *Lactococcus lactis* subsp. *lactis*, and is sold as the commercial extract Nisaplin® Natural Antimicrobial (Danisco). Nisin has an unusually broad antimicrobial spectrum for a bacteriocin, being active against most Gram-positive bacteria (e.g. species of *Bacillus*, *Clostridium*, *Listeria*, lactic acid bacteria). It is not normally effective against Gram-negative bacteria, yeasts or moulds. Nisin is allowed as a food preservative worldwide but its levels of use and approved food applications are strictly regulated, varying from country to country.

Other bacteriocins have since been discovered with potential as food preservatives, e.g. pediocin, lacticin, sakacin, lactococcin, enterococin, plantaricin, leucocin. These are also active, although usually with a more narrow spectrum, against Gram-positive bacteria. Their food use is at present restricted to production of the bacteriocin *in situ*, i.e. by growth of the producer organism within the food.

Food safety and prevention of food spoilage is an ever present concern worldwide, particularly with the increasing trend for convenience foods such as ready to eat meals, soups, sauces or snacks. Spoilage of food is a major economic problem for the food manufacturer. Food manufacturers need to protect the health and safety of the public by delivering products that are safe to eat. Such food must have a guaranteed shelf life, either at chilled or ambient temperature storage. Consumers prefer good tasting food of high quality - this is difficult to achieve with chemical preservatives, harsh heating

regimes and other processing measures. Food safety and protection is best achieved with a multiple preservation system using a combined approach of milder processing and natural preservatives. Foodborne micro-organisms are also less able to adapt and grow in food preserved with different preservative measures.

There is much concern about food safety and the growth of food pathogens such as *Listeria monocytogenes*. This particular pathogen can grow at low temperatures, which are often used as an additional preservative measure. Foodborne pathogens can sometimes adapt to different preservatives and storage conditions, thus a combination of preservative measures can be more successful than individual measures..

Cooked meat joints are new generation, convenience products now on offer to consumers. The preparation of these meat joints usually involves injection or tumbling of the raw meat in polyphosphate brine to increase the meat's tenderness, moistness and volume. The meat is then cooked before distribution to retail outlets and its subsequent consumer purchase and consumption.

The majority of processes for these meats now involve the 'cook-in' system in which the meat is cooked in plastic bags or film. Whole joints may be de-boned, pumped with polyphosphate brine and tumbled or massaged for a short period. This distributes the brine evenly and also achieves a layer of exudate on the surface that helps the plastic packaging to adhere closely to the meat surface. Large joints are usually gas or vacuum-packaged into plastic bags. These cooked meat products are often considered to be of good quality and healthy, since they may be low in fat with minimal salt content. They may not necessarily be re-heated by the purchaser prior to consumption.

These minimally processed products rely on refrigeration to ensure stability and safety of the cooked meat during shelf life, which may be as long as 90 days (Varnam and Sutherland, 1995). Spoilage of the cooked meats, if post-processing contamination is not a factor, would be due to the Gram-positive heat-resistant bacteria *Bacillus* and *Clostridium*, particularly if the meat is exposed to temperatures above 7 °C. Spoilage due to these organisms can be rapid if the meat is exposed to temperatures as high as 15 °C or above. If the meat has not been sufficiently cooked, *Enterococcus* or heat-resistant *Lactobacillus* species may survive, many of which can grow at refrigeration temperatures. If the product has been handled after cooking then re-packaged and vacuum-packed, spoilage is often associated with *Lactobacillus*, *Leuconostoc* or

Carnobacterium. *Brochothrix thermosphacta*, another Gram-positive bacterium, can also cause problems. Gram-negative bacteria will only be a problem in unpackaged cooked meats, or those that are packed in air-permeable film. Moulds may develop on cooked meat joints that have been exposed to air and whose surfaces have dried out. There is also concern over post-processing contamination and growth of *Listeria monocytogenes*, a foodborne pathogen that can grow at refrigeration temperatures. It would be a benefit to both the public in terms of safety and manufacturers in terms of economics and reputation, if an effective preservative could be somehow applied to the surface layer of the cooked meat.

Raw, whole muscle meat is also being increasingly sold as a chilled convenience meat product that is ready prepared and tenderised for the consumer to cook. The meat is usually covered with a marinade then vacuum-packed in a clear pouch. The marinade may be applied and simply left to soak into the meat surface, or the meat may be tumbled in the marinade to increase its tenderising effect and penetration. This vacuum-packed, marinated fresh meat can be kept for up to 28 days at refrigeration temperatures before purchase by the consumer and subsequent cooking at home. These meat products are considered value-added fresh meats and cover a wide range of raw meats (pork, chicken, beef, ground beef, steaks, diced meats, joints, etc.). The combination of the acidic nature of the marinade and the lack of oxygen in the vacuum-packed pouches means that Gram-positive lactic acid bacteria are associated with spoilage of these products (Susiluoto et al. 2003).

Nisin is a natural preservative that has been used safely in food for nearly 50 years. It is effective against Gram-positive bacteria including lactic acid bacteria, *Brochothrix thermosphacta*, *Listeria monocytogenes*, *Bacillus* and *Clostridium*. As the spoilage associated with both the meat products described above, is usually caused by Gram-positive bacteria, nisin could be considered as part of a preservative system to guarantee or extend shelf life. However the environment of both meat products is not favourable to nisin stability or activity. Brine and polyphosphate solutions used to inject raw meat are usually at alkaline pH. Nisin stability is optimum at pH 3 (Davies et al. 1998). The cooking process, particularly at high or neutral pH conditions, would lead to significant nisin degradation. In raw meat, nisin is vulnerable to degradation by proteases. A more specific concern is the inactivation of nisin in raw meat by the formation of an adduct with glutathione in an enzyme-mediated reaction (Rose et al. 1999, 2002, 2003).

Numerous prior art teachings have discussed potential uses of nisin in foodstuffs. Examples are:

- Caserio et al. (1979) describes research on the use of nisin in cooked, cured meat products. Mortadella, wurstel sausage, prosciutto. The target organisms: *Staphylococcus*, sulphate-reducing clostridia. Prosciutto had nisin injected with brine after dissolution in dilute lactic acid.
- Gola (1962) incorporated nisin into the gelatine for canning of large hams. In the first experiment, brines for injection were acidified to facilitate nisin solubility.
- Taylor & Somers (1985) evaluate the antibotulinal effectiveness of nisin in bacon. Nisin was included in brine formulation injected into pork belly.
- Usborne et al. (1986) discusses sensory evaluation of nisin-treated bacon. Nisin was added to brine pumping solution before injection into the bacon.
- US 2003/0108648 relates to compositions having bacteriostatic and bactericidal activity against bacterial spores and vegetative cells and process for treating foods therewith.
- US 6207210 relates to broad-range antibacterial composition and process of applying to food surfaces
- EP0770336 describes injection of meat trimmings/brine solution in which a starter culture has produced a bacteriocin.
- Article found at http://www.nal.usda.gov/fsrio/ppd/ars010f.htm on work at Meat Research Unit, MARC mentions a presentation on 'antibacterial properties of injectable beef marinades'.
- WO2003/11058 relates to food preservation formulation comprising compound(s) derived from natural sources. Natural compounds are formulated and application to a food and irradiation at < 3kGy results in decrease of microflora compared to non-irradiated controls. Nisin is a preferred compound.
- US 2003/0108648 teaches nisin as part of a combination for marinades.

The above extensive prior art does not address or solve the problems of protection of antimicrobial materials such as nisin from environments, such as those in meat products, which are not favourable to the stability or activity of the antimicrobial material.

The present invention alleviates the problems of the prior art.

In one aspect the present invention provides a process for introducing an antimicrobial

material into a foodstuff comprising (i) providing the antimicrobial material in an encapsulated form comprising a core of antimicrobial material and shell of encapsulating material, (ii) introducing encapsulated antimicrobial material into the foodstuff by (a) injecting the encapsulated antimicrobial material into the foodstuff or (b) tumbling the encapsulated antimicrobial material with the foodstuff.

In one aspect the present invention provides a foodstuff prepared by a process for introducing an antimicrobial material into a foodstuff comprising (i) providing the antimicrobial material in an encapsulated form comprising a core of antimicrobial material and shell of encapsulating material, (ii) introducing encapsulated antimicrobial material into the foodstuff by (a) injecting the encapsulated antimicrobial material into the foodstuff or (b) tumbling the encapsulated antimicrobial material with the foodstuff.

In one aspect the present invention provides a foodstuff obtainable by a process for introducing an antimicrobial material into a foodstuff comprising (i) providing the antimicrobial material in an encapsulated form comprising a core of antimicrobial material and shell of encapsulating material, (ii) introducing encapsulated antimicrobial material into the foodstuff by (a) injecting the encapsulated antimicrobial material into the foodstuff or (b) tumbling the encapsulated antimicrobial material with the foodstuff.

In one aspect the present invention provides an antimicrobial material in an encapsulated form, comprising a core of antimicrobial material and shell of encapsulating material, wherein the shell of encapsulating material is impermeable to the antimicrobial material.

The term "encapsulated" is well known in the art. Encapsulation can be defined as the technology of packaging a substrate (solids, liquids, gases) within another material. In the encapsulate the material which has been entrapped is termed the core material or the internal phase while the encapsulating material is referred to as the coating, the shell material or the carrier. Such encapsulated materials are also commonly referred to as core/shell materials.

Aspects of the invention are defined in the appended claims.

We have found that by providing the present antimicrobial materials in an encapsulated form the materials may be protected from environments, such as those in meat products, which are not favourable to the stability or activity of the antimicrobial material. Moreover,

by injecting the encapsulated antimicrobial material into the foodstuff or tumbling the encapsulated antimicrobial material with the foodstuff, the encapsulated antimicrobial material may be effectively introduced into the foodstuff. We have found the injection particularly advantageous and surprising. Prior art processes have directly injected non-encapsulated antimicrobial materials such as nisin into food products. We have found that a "shell" may be provided on the antimicrobial material which is capable of withstanding the demanding physical forces exerted on the encapsulated antimicrobial material during injection. In particular injection exerts high pressures and yield stress on the material to be injected. We have also found that a "shell" may be provided on the antimicrobial material which is capable of protecting the antimicrobial material from adverse conditions and/or allows sustained/controlled release.

The present invention provides a process for delivering an antimicrobial material and an anti-microbial material per se which is resistant to unwanted degradation and which may be released to provide a long term antimicrobial effect.

For ease of reference, these and further aspects of the present invention are now discussed under appropriate section headings. However, the teachings under each section are not necessarily limited to each particular section.

PREFERRED ASPECTS

ANTIMICROBIAL MATERIAL

In one preferred aspect the antimicrobial material is an antibacterial material.

In one preferred aspect the antimicrobial material is a bacteriocin.

The antimicrobial material, such as a bacteriocin, may typically be selected from materials (bacteriocins) that can be used as preservatives in food.

Preferably the antimicrobial material is selected from lanthionine containing bacteriocins, *Lactococcus*-derived bacteriocins, *Streptococcus*-derived bacteriocins, *Pediococcus*-derived bacteriocins, *Lactobacillus*-derived bacteriocins, *Carnobacterium*-derived bacteriocins, *Leuconostoc*-derived bacteriocins, *Enterococcus*-derived bacteriocins and mixtures thereof.

Preferably the antimicrobial material is at least nisin.

Preferably the antimicrobial material consists of nisin.

Nisin is a lanthionine-containing bacteriocin (US 5691301) derived from *Lactococcus lactis* subsp. *lactis* (formerly known as *Streptococcus-lactis*) (US 5573801). In a preferred aspect of the present invention the bacteriocin used in the present invention is at least nisin.

As discussed in US 5573801 nisin is a polypeptide bacteriocin produced by the lactic acid bacteria, *Lactococcus lactis* subsp. *lactis* (formerly known as *Streptococcus lactis* Group N).

Nisin is reportedly a collective name representing several closely related substances which have been designated nisin compounds A, B, C, D and E (De Vuyst, L. and Vandamme, E. J. 1994. Nisin, a lantibiotic produced by *Lactococcus lactis* subsp. *lactis*: properties, biosynthesis, fermentation and applications. In: Bacteriocins of lactic acid bacteria. Microbiology, Genetics and Applications. Eds.: De Vuyst and Vandamme. Blackie Academic and Professional, London). . The structure and properties of nisin are also discussed in the article by E. Lipinska, entitled "Nisin and Its Applications", The 25th Proceedings of the Easter School in Agriculture Science at the University of Nottingham, 1976, pp. 103-130 (1977), which article is hereby incorporated by reference. In 1969 the FAO/WHO Joint Expert Committee on Food Additives set specifications for the purity and identity of nisin (FAO/WHO Joint Expert Committee on Food Additives. 1969. Specifications for identity and purity of some antibiotics. 12th Report. WHO Technical Report Series No. 430). This committee recognised nisin as a safe and legal preservative based on extensive toxicological testing. Nisin has the food additive number E234 and is classed as GRAS (Generally Recognised As Safe) (Food and Drug Administration. 1988. Nisin preparation: Affirmation of GRAS status as a direct human ingredient. Federal Regulations 53: 11247). The international activity unit (IU hereinafter) was defined as 0.001 mg of an international nisin reference preparation. Nisaplin® Natural Antimicrobial is the brand name for a nisin concentrate containing 1 million IU per g, which is commercially available from Danisco.

Nisin is an acknowledged and accepted food preservative with a long history of safe,

effective food use. There have been several reviews of nisin, e.g. Hurst 1981; 1983; Delves-Broughton, 1990; De Vuyst and Vandamme, 1994; Thomas *et al.* 2000; Thomas & Delves-Broughton, 2001). Nisin was discovered over 50 years ago and the first commercial preparation, made in 1953, was Nisaplin®. Nisin has several characteristics that make it particularly suitable as a food preservative. It has undergone extensive toxicological testing to demonstrate its safety. It is heat-stable, acid-stable and effective against a broad spectrum of Gram-positive bacteria. It is not normally effective against Gram-negative bacteria, yeasts or moulds but activity against Gram-negative bacteria and yeasts has been reported in the presence of chelating agents (PCT/US 8902625. WO 89/12399). Nisin is an effective preservative in pasteurised and heat-treated foods (e.g. processed cheese, cheese, pasteurised milks, dairy desserts, cream, mascarpone and other dairy products, puddings such as semolina, tapioca etc., pasteurised liquid egg, pasteurised potato products, soy products, crumpets, pikelets, flapjacks, processed meat products, beverages, soups, sauces, ready to eat meals, canned foods, vegetable drinks) and low acid foods such as salad dressings, sauces, mayonnaise, beer, wine and other beverages.

Although some loss of activity may be expected when used with processed foods, this may be ameliorated e.g. by increasing the amount of nisin applied. Effective levels of nisin to preserve foodstuffs reportedly range from 25-500 IU/g or more. Other effective levels would be appreciated by one skilled in the art. For example levels of 50-400 IU/g may be utilised.

Since the discovery of the first bacteriocin, nisin, many other bacteriocins have now been found (Hoover, 1993; Ray & Daeschel, 1994; Axelsen, 1998; Naidu, 2000; Ray *et al.* 2001; Ray & Miller, 2003). The bacteriocin pediocin, produced by *Pediococcus pentosaceus*, *P. acidilactici*, or *Lactobacillus plantarum*, may be used in the present invention. Like nisin, different structures of pediocin have been described. At present pediocin and other bacteriocins are not allowed as food additives but their antibacterial activity can be achieved by production of the bacteriocin *in situ*, as a consequence of the growth of the producer organism in the food. This is the purpose of commercial protective cultures such as HOLDBAC™ Listeria (Danisco). Pediocin has a more narrow antimicrobial spectrum compared to nisin, but there is much interest in its food safety ability to kill, prevent or control the growth of the food pathogen *Listeria monocytogenes* (Ray & Miller, 2000). Other bacteriocins may be used in the present invention, including those named generally as divercin, leucocin, mesentericin, sakacin, curvacin, bavaricin,

acidocin, bifidocin, carnobacteriocin, pisicocin, piscicolin, mundticin, enterocin, thermophilin, lacticin, plantaricin, lactococcin, dricin, diplococcin, mesenterocin, leuconosin, carnosin, acidophilin, lactacin, brevicin, lactocin, helevticin, reutericin, propionicin.

MICROORGANISM

As discussed herein the present invention may prevent and/or inhibit the growth of, and/or kill a micro-organism in a material. This may be slowing or arresting a micro-organism, such a bacteria, or by killing the micro-organism present on contact with the present composition.

In one aspect the antimicrobial material is present in an amount to provide a microbicidal or microbiostatic effect.

In one aspect the bacteriocin and the extract are present in an amount to provide a microbicidal or microbiostatic effect.

In a highly preferred aspect the microbicidal or microbiostatic effect is a bactericidal or bacteriostatic effect.

It is advantageous for the bactericidal or bacteriostatic effect to be in respect of Gram-positive bacteria and Gram-negative bacteria. Preferably the bactericidal or bacteriostatic effect is in respect of Gram-positive bacteria.

In a preferred aspect the bactericidal or bacteriostatic effect is in respect of an organism selected from Gram-positive bacteria associated with food spoilage or foodborne disease including *Bacillus* species, *Bacillus subtilis, Bacillus cereus, Listeria* species, *Listeria monocytogenes*, lactic acid bacteria, lactic acid spoilage bacteria, *Lactobacillus species*, *Staphylococcus aureus, Clostridium* species, *C. sporogenes, C. tyrobutyricum* and *C. botulinum* (when the antimicrobial material is recognised as effective against *C. botulinum* or is part of a system effective against *C. botulinum*).

In a preferred aspect the bactericidal or bacteriostatic effect of the invention in combination with a chelating agent is in respect of an organism selected from other micro-organisms associated with food spoilage or foodborne disease, including yeasts,

moulds and Gram-negative bacteria including *Escherichia coli*, *Salmonella* species, and *Pseudomonas* species.

In a preferred aspect the bactericidal or bacteriostatic effect is in respect of lactic acid bacteria such as *Lactobacillus*, *Leuconostoc*, *Carnobacterium*, and *Enterococcus*; *Listeria monocytogenes*, spore forming heat resistant bacteria such as *Bacillus* and *Clostridium*; and *Brochothrix thermosphacta*.

In a preferred aspect the bactericidal or bacteriostatic effect is in respect of *Lactobacillus*, *Leuconostoc*, *Carnobacterium*, *Enterococcus*, *Listeria monocytogenes*, *Bacillus*, *Clostridium*; and *Brochothrix thermosphacta*.

In a preferred aspect the bacteriostatic effect is in respect of *Bacillus cereus*.

In a preferred aspect the bactericidal or bacteriostatic effect is in respect of *Listeria monocytogenes*.

ENCAPSULATED ANTIMICROBIAL MATERIAL

In a preferred aspect the encapsulated antimicrobial material is a particulate form.

Particle size may be important either in the injection aspect of the present invention or the tumbling aspect. The choice of particle size, for example to a particular maximum average particle size, may assist in the introduction of the encapsulated antimicrobial material into the foodstuff. We have found that in the injection aspect of the particle size is particularly important. The particle size, and in particular the maximum average particle size, may determine the likelihood that the shell of the encapsulated antimicrobial material will withstand an injection process.

In a preferred aspect the encapsulated antimicrobial material has an average particle size of less than 500µm, preferably less than 300µm, preferably less than 250µm, preferably less than 150µm, preferably from 50 to 150µm. In some aspects the encapsulated antimicrobial material has an average particle size of less than 100µm, or less than 50µm, or less than 25µm.

As discussed above an aim of the present invention is to provide for the introduction of

the antimicrobial material into the foodstuff in a form protected from degradation or inactivation. However, the antimicrobial material should of course be released when required so as to provide the antimicrobial effect which is its purpose. Thus in one preferred aspect the shell is selected to provide sustained release of the antimicrobial material from the encapsulated antimicrobial material.

In one aspect of the present invention the shell is selected to provide sustained release of the antimicrobial material from the encapsulated antimicrobial material or to provide release under predetermined conditions. Suitable predetermined temperature conditions are: greater than 50°C, preferably greater than 60°C, preferably greater than 70°C, preferably greater than 72°C, preferably greater than 75°C, preferably from 72 to 78°C.

In one aspect of the present invention the shell is selected to prevent, reduce or inhibit degeneration or inactivation of the antimicrobial material. Preferably the degeneration which is to be prevented is by one or more factors selected from heat degradation, pH induced degradation, protease degradation and glutathione adduct formation.

Thus in one further aspect the present invention provides use of an encapsulating material for the prevention, reduction or inhibition of the degeneration or inactivation of an antimicrobial material. Preferably the degeneration to be prevented is by one or more factors selected from heat degradation, pH induced degradation, protease degradation and glutathione adduct formation.

The shell is or comprises or may be formed from any suitable material. Preferred materials are selected from fats, emulsifiers, waxes (animal, vegetable, mineral or synthetic), liposome-forming lipids (such as glycerophospholipids and sterols), hydrocolloids, natural or synthetic polymers and mixtures thereof. Preferred materials are materials that are brine-insoluble or can be rendered brine insoluble by crosslinking, sintering or other means.

Preferably the glycerophospholipids are selected from phosphatidycholines, phosphatidyethanolamines and phosphatidylglycerols.

Preferably the sterols are selected from cholesterol, ergosterol, lanosterol, and stigmasterol.

Preferably the fat is a triglyceride, more preferably a vegetable triglyceride.

Preferably the emulsifier is selected from polysorbates, monoglycerides, diglycerides, acetic acid esters of mono-diglycerides, tartaric acid esters of mono-diglycerides and citric acid esters of mono-diglycerides.

Preferably the hydrocolloid is cross linked or gelled.

The cross-linking of the hydrocolloids may be is carried out by using cross-linking agents or by a variety of mechanisms. If the hydrocolloid is a protein or polysaccharide bearing amino groups, it can be cross-linked by using dialdehydes, such as glutaraldehyde. If the hydrocolloid is a polysaccharide, such as sodium alginate, gellan gum or pectin, it can be cross-linked with multivalent ions, such as calcium or magnesium. The cross-linking can also be carried out by other mechanisms, such as heating, pH adjustment, applying pressure or by enzymatic cross-linking. Proteins, for example, can be cross-linked by subjecting a protein to a high pressure, preferably from 5 to 200 bar, and/or by subjecting a protein to a temperature which is above the denaturation temperature of the protein. The enzymatic cross-linking of proteins can be carried out for example with transglutamidase. Based on the hydrocolloid used, a person skilled in the art is able to decide which method of gelling or cross-linking is used.

Preferably the hydrocolloid is selected from carrageenan.

In one aspect the hydrocolloid is selected from alginate, carrageenan, carboxymethyl cellulose (CMC), guar gum, locust bean gum (LBG), xanthan gum, microcrystalline cellulose (MCC), methyl cellulose (MC), cellulose ethers including hydroxy propyl methyl cellulose (HPMC), pectin, starch including native and modified starch, pregelatinated starch and non-pregelatinated starch, including starch from corn, potato, tapioca, wheat, and rice, gelatin, agar, and combinations thereof.

In one aspect the hydrocolloid is brine-insoluble, particularly at the temperature of use, or a hydrocolloids rendered insoluble by crosslinking or gelling.

Preferably the natural or synthetic polymer is selected from shellac, polyvinyl acetate, polymethyl-metacrylate and its derivatives, any brine-insoluble polymers.

In one further preferred aspect the shell is or comprises or may be formed from the group comprising fats, oils, waxes, resins, emulsifiers or mixtures thereof, which are preferably food-grade. Preferably the hydrophobic shell matrix is selected from the group comprising animal oils and fats, fully hydrogenated vegetable or animal oils, partially hydrogenated vegetable or animal oils, unsaturated, hydrogenated or fully hydrogenated fatty acids, unsaturated, partially hydrogenated or fully hydrogenated fatty acid monoglycerides and diglycerides, unsaturated, partially hydrogenated or fully hydrogenated esterified fatty acids of monoglycerides or diglycerides, unsaturated, partially hydrogenated or fully hydrogenated free fatty acids, other emulsifiers, animal waxes, vegetable waxes, mineral waxes, synthetic waxes, natural and synthetic resins and mixtures thereof.

Animal oils and fats are such as, but not restricted to, beef tallow, mutton tallow, lamb tallow, lard or pork fat, sperm oil. Hydrogenated or partially hydrogenated vegetable oils are such as, but not restricted to, canola oil, cottonseed oil, peanut oil, corn oil, olive oil, soybean oil, sunflower oil, safflower oil, coconut oil, palm oil, linseed oil, tung oil and castor oil. Free fatty acids are such as, but not restricted to, stearic acid, palmitic acid and oleic acid. Other emulsifiers are such as, but not restricted to, polyglycerol esters, sorbitan esters of fatty acids. Animal waxes are such as, but not restricted to, beeswax, lanolin, shell wax or Chinese insect wax. Vegetable waxes are such as, but not restricted to, carnauba, candelilla, bayberry or sugarcane waxes. Mineral waxes are such as, but not restricted to, paraffin, microcrysalline petroleum, ozocerite, ceresin or montan. Synthetic waxes are such as, but not restricted to, low molecular weight polyolefin, polyol ether-esters and Fisher-Tropsch process synthetic waxes. Natural resins are such as rosin, balsam, shellac and zein.

In one further preferred aspect the shell is or comprises or may be formed from the group comprising hydrocolloids, sodium alginate, gum arabic, gellan gum, starch, modified starch, guar gum, agar gum, pectin, amidified pectin, carrageenan, xanthan, gelatine, chitosan, mesquite gum, hyaluronic acid, cellulose derivatives such as cellulose acetate phtalate, hydroxy propyl methylcellulose (HPMC), methyl cellulose, ethyl cellulose and carboxy methyl cellulose (CMC), methyl acrylic copolymers, such as Eudragit®, psyllium, tamarind, xanthan, locust bean gum, whey protein, soy protein, sodium caseinate, any food-grade protein, shellac, zein, any synthetic or natural water-soluble polymers, any water-insoluble microparticles, such as silicone dioxide, titanium dioxide, synthetic or natural food-grade polymer beads and mixtures thereof.

The encapsulated antimicrobial material may be prepared by any suitable process. In one preferred aspect the encapsulated antimicrobial material is prepared by or is obtainable by a process selected from spray cooling, and fluidised bed coating.

Further preferred aspects include

(a) spray cooling in fats, waxes or emulsifiers

(b) fluidised bed coating with acid-stable shellac coating, fats, waxes, or emulsifiers, or any other hydrophobic and/or acid stable coating

(c) complex or simple co-acervation in cross-linked hydrocolloids.

In one preferred aspect the shell of the encapsulated antimicrobial material is capable of withstanding injection into the foodstuff.

In one preferred aspect the shell of the encapsulated antimicrobial material is capable of withstanding a pressure of greater than 1.5 bar, for example. greater than 2.0 bar, for example greater than 3.0 bar.

In one preferred aspect the shell of the encapsulated antimicrobial material is capable of withstanding a shear force of greater than that typically encountered during injection.

As discussed herein the shell of the encapsulated antimicrobial material may be selected to provide sustained release of the antimicrobial material from the encapsulated antimicrobial material or to provide release under predetermined conditions. Furthermore the shell of the encapsulated antimicrobial material may be selected to prevent, reduce or inhibit degeneration or inactivation of the antimicrobial material. Preferably the degeneration which is to be prevented is by one or more factors selected from heat degradation, pH induced degradation, protease degradation and glutathione adduct formation.

We have found that provision of an encapsulated antimicrobial material in which the shell is selected to provide sustained release of the antimicrobial material from the encapsulated antimicrobial material or to provide release under predetermined conditions is advantageous irrespective of the manner in which the encapsulated antimicrobial material is contacted with a foodstuff. For example the encapsulated antimicrobial material may be contacted with a foodstuff (or other material) by means other than

injection or tumbling. In other words, we have provided an encapsulated antimicrobial material in which antimicrobial material is released in a sustained manner or when the encapsulated antimicrobial material is placed under predetermined conditions.

Thus in a further aspect (the "encapsulated material" aspect) the present invention provides an antimicrobial material in an encapsulated form, comprising a core of antimicrobial material and shell of encapsulating material, wherein the shell of encapsulating material is impermeable to the antimicrobial material.

This preservation system has the benefits of maximising the potential of the antimicrobial material (such as nisin) which may added to the food, offering no taste impact, cheapness, ease- of-use, ease-of-manufacture and stability. In some aspects of the invention it may also be described as "natural" for food labelling purposes.

In this and other aspects of the invention, by the term "encapsulated" it is meant the packaging of solid particles or liquid droplets of active ingredient (or particles or droplets containing the active ingredient) within a secondary material so as to fully surround the solid particles or liquid droplets with a protective or functional shell material. This contrasts with the loose use of the term encapsulated to refer to simple coating. For example Cahill et al. teaches the coating of nisin with a porous matrix of alginate. External material can freely diffuse in the alginate matrix and the coated nisin can easily diffuse out through the large pores of the matrix. This is not "encapsulation" in the present sense.

Each of the preferred aspects described herein are applicable to the encapsulated material aspect of the invention. Particularly preferred aspects include

- the antimicrobial material is an antibacterial material.
- the antimicrobial material is a bacteriocin.
- the antimicrobial material is at least nisin.
- the shell is selected to provide sustained release of the antimicrobial material from the encapsulated antimicrobial material.
- the shell is selected to prevent, reduce or inhibit degeneration or inactivation of the antimicrobial material.
- shell is selected to prevent, reduce or inhibit degeneration or inactivation of the antimicrobial material by one or more factors selected from heat degradation, pH

induced degradation, protease degradation and glutathione adduct formation.

- the shell is selected to release the antimicrobial material from the encapsulated antimicrobial material on contact with a foodstuff, preferably the foodstuff is a marinade.
- the antimicrobial material provides a bactericidal or bacteriostatic effect in respect of *Listeria monocytogenes*.
- the encapsulated material is utilised in the protection of microbial spoilage of a foodstuff selected from raw meat products, cooked meat products, raw seafood products, cooked seafood products, raw poultry products and cooked poultry products.
- the encapsulated material is utilised in the protection of microbial spoilage of a raw poultry product.

Highly preferred aspects of all aspects of the invention and in particular the encapsulated material aspect include

- the antimicrobial material is at least nisin.
- the shell is selected to prevent, reduce or inhibit degeneration or inactivation of the antimicrobial material by one or more factors selected from heat degradation, pH induced degradation, protease degradation and glutathione adduct formation.
- the antimicrobial material provides a bactericidal or bacteriostatic effect in respect of *Listeria monocytogenes*.
- the encapsulated material is utilised in the protection of microbial spoilage of a foodstuff selected from raw meat products, cooked meat products, raw seafood products, cooked seafood products, raw poultry products and cooked poultry products.
- the encapsulated material is utilised in the protection of microbial spoilage of a raw poultry product.
- the antimicrobial material is at least nisin; and the shell is selected to prevent, reduce or inhibit degeneration or inactivation of the antimicrobial material by one or more factors selected from heat degradation, pH induced degradation, protease degradation and glutathione adduct formation.
- the antimicrobial material is at least nisin; and the antimicrobial material provides a bactericidal or bacteriostatic effect in respect of *Listeria monocytogenes*.
- the antimicrobial material is at least nisin; and the encapsulated material is utilised in the protection of microbial spoilage of a foodstuff selected from raw meat products,

cooked meat products, raw seafood products, cooked seafood products, raw poultry products and cooked poultry products.

- the shell is selected to prevent, reduce or inhibit degeneration or inactivation of the antimicrobial material by one or more factors selected from heat degradation, pH induced degradation, protease degradation and glutathione adduct formation; and the antimicrobial material provides a bactericidal or bacteriostatic effect in respect of *Listeria monocytogenes*.
- the shell is selected to prevent, reduce or inhibit degeneration or inactivation of the antimicrobial material by one or more factors selected from heat degradation, pH induced degradation, protease degradation and glutathione adduct formation; and the encapsulated material is utilised in the protection of microbial spoilage of a foodstuff selected from raw meat products, cooked meat products, raw seafood products, cooked seafood products, raw poultry products and cooked poultry products.
- the antimicrobial material provides a bactericidal or bacteriostatic effect in respect of *Listeria monocytogenes*; and the encapsulated material is utilised in the protection of microbial spoilage of a foodstuff selected from raw meat products, cooked meat products, raw seafood products, cooked seafood products, raw poultry products and cooked poultry products.
- the antimicrobial material is at least nisin; and the shell is selected to prevent, reduce or inhibit degeneration or inactivation of the antimicrobial material by one or more factors selected from heat degradation, pH induced degradation, protease degradation and glutathione adduct formation; and the antimicrobial material provides a bactericidal or bacteriostatic effect in respect of *Listeria monocytogenes*.
- the antimicrobial material is at least nisin; and the shell is selected to prevent, reduce or inhibit degeneration or inactivation of the antimicrobial material by one or more factors selected from heat degradation, pH induced degradation, protease degradation and glutathione adduct formation; and the encapsulated material is utilised in the protection of microbial spoilage of a foodstuff selected from raw meat products, cooked meat products, raw seafood products, cooked seafood products, raw poultry products and cooked poultry products.
- the antimicrobial material is at least nisin; and the antimicrobial material provides a bactericidal or bacteriostatic effect in respect of *Listeria monocytogenes*; and the encapsulated material is utilised in the protection of microbial spoilage of a foodstuff selected from raw meat products, cooked meat products, raw seafood products,

cooked seafood products, raw poultry products and cooked poultry products.

- the shell is selected to prevent, reduce or inhibit degeneration or inactivation of the antimicrobial material by one or more factors selected from heat degradation, pH induced degradation, protease degradation and glutathione adduct formation; and the antimicrobial material provides a bactericidal or bacteriostatic effect in respect of *Listeria monocytogenes*; and the encapsulated material is utilised in the protection of microbial spoilage of a foodstuff selected from raw meat products, cooked meat products, raw seafood products, cooked seafood products, raw poultry products and cooked poultry products.
- the antimicrobial material is at least nisin; and the shell is selected to prevent, reduce or inhibit degeneration or inactivation of the antimicrobial material by one or more factors selected from heat degradation, pH induced degradation, protease degradation and glutathione adduct formation; and the antimicrobial material provides a bactericidal or bacteriostatic effect in respect of *Listeria monocytogenes*; and the encapsulated material is utilised in the protection of microbial spoilage of a foodstuff selected from raw meat products, cooked meat products, raw seafood products, cooked seafood products, raw poultry products and cooked poultry products.

FOODSTUFF

Many foodstuffs may be protected by the present invention. Typical foodstuffs are raw meat, cooked meat, raw poultry products, cooked poultry products, raw seafood products, cooked seafood products, [raw or cooked meat, poultry and seafood products], ready to eat meals, pasta sauces, pasteurised soups, mayonnaise, salad dressings, marinades, oil-in-water emulsions, margarines, low fat spreads, water-in-oil emulsions, dairy products, cheese spreads, processed cheese, dairy desserts, flavoured milks, cream, fermented milk products, cheese, butter, condensed milk products, ice cream mixes, soya products, pasteurised liquid egg, bakery products (such as crumpets), confectionery products, fruit products, and foods with fat-based or water-containing fillings.

In one preferred aspect the foodstuff is a bakery product.

In one preferred aspect the foodstuff is selected from raw meat, cooked meat, raw poultry products, cooked poultry products, raw seafood products, cooked seafood

products [raw or cooked meat, poultry and seafood products] and raw or cooked foodstuffs prone to surface bacterial growth.

In one preferred aspect the foodstuff is raw meat.

In one preferred aspect the foodstuff is selected from raw meat products, cooked meat products, raw seafood products, cooked seafood products, raw poultry products and cooked poultry products.

In one preferred aspect the foodstuff is selected from raw poultry products and cooked poultry products.

In one preferred aspect the foodstuff is a raw poultry product.

In one preferred aspect the foodstuff comprises whole meat muscle.

It will be appreciated by one skilled in the art that by the term "cooked" product it is meant a food product which has undergone some degree of cooking (either partial or complete). It will appreciated by one skilled in the art that the cooked products of the present invention may be subjected to further cooking after contacting with the encapsulated material of the present invention. In one preferred aspect the cooked products of the present invention are subjected to further cooking after contacting with the encapsulated material of the present invention. This subsequent cooking provides for release of the antimicrobial material from the encapsulated product and consequently activation of the antimicrobial material protective effect.

ADDITIONAL COMPONENTS

Typically the encapsulated antimicrobial material will not be introduced into the foodstuff alone. Thus in one aspect the encapsulated antimicrobial material is introduced into the foodstuff in a carrier. Preferably the carrier is or comprises brine.

The density of the encapsulated antimicrobial material should match the density of the carrier (such as brine) to avoid separation or sedimentation of the encapsulated antimicrobial material, preventing even distribution of encapsulated antimicrobial material during injection or tumbling. Thus in a preferred aspect the carrier and the encapsulated

antimicrobial material have substantially the same density.

Matching the density of the carrier and the encapsulated antimicrobial material may be achieved by careful selection of carrier and encapsulated antimicrobial material. Alternatively it may be achieved by modification of the encapsulated antimicrobial material to have substantially the same density as the carrier, or by modification of the carrier to have substantially the same density as the encapsulated antimicrobial material. The encapsulated antimicrobial material may be modified by contacting the encapsulated antimicrobial material with oil, such as a brominated oil. The carrier may be modified by inclusion of an additional component such as xanthum gum.

The carrier may contain one or more additional components. However, in some aspects the carrier contains no additional components or contains no additional components that materially affect the properties of the composition.

In one preferred aspect the carrier further comprises an emulsifier. Preferably the emulsifier is selected from polyoxy-ethylene sorbitan esters (E432-E436) otherwise known as polysorbates (e.g. Tween 80, Tween 20), monoglycerides, diglycerides, acetic acid esters of mono-diglycerides, tartaric acid esters of mono-diglycerides and citric acid esters of mono-diglycerides.

The encapsulated antimicrobial material may contain one or more additional components. However, in some aspects the encapsulated antimicrobial material contains no additional components or contains no additional components that materially affect the properties of the composition.

In one preferred aspect the encapsulated antimicrobial material further comprises an extract obtained from or obtainable from a plant of the Labiatae family. Optionally in this aspect and particularly when the antimicrobial material consists of nisin, the composition comprises carvacrol in an amount of less than 0.075wt.% based on the composition and carvone in an amount of less than 15wt.% based on the composition. Compositions comprising an antimicrobial material and an extract obtained from or obtainable from a plant of the Labiatae family are discussed in our British Patent Application No. 0323335.0 Each of the teachings of GB 0323335.0 are applicable to the present system.

In one aspect the extract used is obtained from a plant of the Labiatae family.

It will be appreciated by one skilled in the art that by the term "extract" or "extracts" it is meant any constituent of the plant which may be isolated from the whole plant.

In one aspect the extract used in the present invention is obtainable from a plant of the Labiatae family. It will be appreciated by one skilled in the art that an extract obtainable from a plant may be obtained from a plant or may be isolated from the plant, identified and then obtained from an alternative source, for example by chemical synthesis or enzymatic production. For example the extract may be produced by a eukaryotic or prokaryotic fermentation, by a process of genetic manipulation. The present applicant have recognised that products present in a plant of the Labiatae family may synergistically increase the activity of a an antimicrobial material, preferably a bacteriocin. These products may be obtained from any source and will fall within the scope of the present invention.

The invention comprises use of a combination of a bacteriocin such as nisin and of the Labiatae plant family, such as rosemary (*Rosmarinus officinalis*) or sage (*Salvia officinalis*) that together give enhanced control of Gram-positive bacteria in a food system. The extracts responsible for synergy in the present invention preferably refer to extracts of the plant family Labiatae that have been selectively extracted ("deodorised extracts") to increase their phenolic diterpene content (such as camosol and carnosic acid), phenolic tripterpene content (such as ursolic acid, betulinic acid and oleanolic acid) or rosmarinic acid content. These deodorised extracts can be distinguished by their high phenolic diterpene content (for example greater than 3.5 wt.%) and their low level (less than 1 wt.%) of flavour-inducing compounds from plant essential oils and oleoresins that are used as flavours or fragrances. Essential oils are typically extracted by simple steam distillation of the plant material.

In one preferred aspect the extract is a deodorised extract. Preferably the (deodorised) extract contains from 1.0 to 70 wt.% phenolic diterpenes, preferably 3.5 to 70 wt.% phenolic diterpenes and less than 1 wt.% essential oil.

In one preferred aspect the extract is selected from phenolic diterpenes, phenolic triterpenes and rosmarinic acid.

In one preferred aspect the extract is or comprises a phenolic diterpene. Preferably the

phenolic diterpene is selected from carnosic acid, carnosol and methylcarnosic acid. Preferably the phenolic diterpene is selected from carnosic acid and carnosol.

In one highly preferred aspect the extract contains one or more phenolic triterpenes. Preferably the phenolic triterpenes are selected from betulinic acid, oleanolic acid, and ursolic acid.

In one preferred aspect is or comprises a phenolic triterpene. Preferably the phenolic triterpene is selected from betulinic acid, oleanolic acid, and ursolic acid.

In one preferred aspect the extract is or comprises rosmarinic acid.

In one preferred aspect the plant of the Labiatae family is selected from rosemary, sage, oregano, marjoram, mint, balm, savoury and thyme. In one preferred aspect the plant of the Labiatae family is selected from rosemary, sage, oregano, marjoram, mint, balm, and savoury. It will be understood that these name cover all species and varieties of plants known by these names.

In one preferred aspect the plant of the Labiatae family is selected from rosemary (*Rosmarinus officinalis* L.), sage (*Salvia officinalis* L.) oregano (*Origanum vulgare* L.), marjoram (*Origanum marjorana* L.), mint (*Mentha* spp.), balm (*Melissa officinalis* L.), savoury (*Satureia hortensis*), thyme (*Thymus vulgaris* L.).

In one preferred aspect the plant of the Labiatae family is selected from rosemary (*Rosmarinus officinalis* L.), sage (*Salvia officinalis* L.) oregano (*Origanum vulgare* L.), marjoram (*Origanum marjorana* L.), mint (*Mentha* spp.), balm (*Melissa officinalis* L.), and savoury (*Satureia hortensis*).

In one preferred aspect the plant of the Labiatae family is rosemary.

In one preferred aspect the encapsulated antimicrobial material further comprises a chelator. Preferably the chelator is selected from EDTA, citric acid, monophosphates, diphosphates, triphosphates and polyphosphates.

Further suitable chelator are taught in US 5573801 and include carboxylic acids, polycarboxylic acids, amino acids and phosphates. In particular, the following

compounds and their salts may be useful:

Acetic acid, Adenine, Adipic acid, ADP, Alanine, B-Alanine, Albumin, Arginine, Ascorbic acid, Asparagine, Aspartic acid, ATP, Benzoic acid, n-Butyric acid, Casein, Citraconic acid, Citric acid, Cysteine, Dehydracetic acid, Desferri-ferrichrysin, Desferri-ferrichrome, Desferri-ferrioxamin E, 3,4-Dihydroxybenzoic acid, Diethylenetriaminepentaacetic acid (DTPA), Dimethylglyoxime, O,O-Dimethylpurpurogallin, EDTA, Formic acid, Fumaric acid, Globulin, Gluconic acid, Glutamic acid, Glutaric acid, Glycine, Glycolic acid, Glycylglycine, Glycylsarcosine, Guanosine, Histamine, Histidine, 3-Hydroxyflavone, Inosine, Inosine triphosphate, Iron-free ferrichrome, Isovaleric acid, Itaconic acid, Kojic acid, Lactic acid, Leucine, Lysine, Maleic acid, Malic acid, Methionine, Methylsalicylate, Nitrilotriacetic acid (NTA), Ornithine, Orthophosphate, Oxalic acid, Oxystearin, B-Phenylalanine, Phosphoric acid, Phytate, Pimelic acid, Pivalic acid, Polyphosphate, Proline, Propionic acid, Purine, Pyrophosphate, Pyruvic acid, Riboflavin, Salicylaldehyde, Salicyclic acid, Sarcosine, Serine, Sorbitol, Succinic acid, Tartaric acid, Tetrametaphosphate, Thiosulfate, Threonine, Trimetaphosphate, Triphosphate, Tryptophan, Uridine diphosphate, Uridine triphosphate, n-Valeric acid, Valine, and Xanthosine.

Many of the above sequestering agents are useful in food processing in their salt forms, which are commonly alkali metal or alkaline earth salts such as sodium, potassium or calcium or quaternary ammonium salts. Sequestering compounds with multiple valencies may be beneficially utilised to adjust pH or selectively introduce or abstract metal ions e.g. in a food system coating. Additional information chelators is disclosed in T. E. Furia (Ed.), CRC Handbook of Food Additives, 2nd Ed., pp. 271-294 (1972, Chemical Rubber Co.), and M. S. Peterson and A. M. Johnson (Eds.), Encyclopaedia of Food Science, pp. 694-699 (1978, AVI Publishing Company, Inc.) which articles are both hereby incorporated by reference.

The terms "chelator" is defined as organic or inorganic compounds capable of forming co-ordination complexes with metals. Also, as the term " chelator" is used herein, it includes molecular encapsulating compounds such as cyclodextrin. The chelator may be inorganic or organic, but preferably is organic.

Preferred chelator are non-toxic to mammals and include aminopolycarboxylic acids and their salts such as ethylenediaminetetraacetic acid (EDTA) or its salts (particularly its di-

and tri-sodium salts), and hydrocarboxylic acids and their salts such as citric acid. However, non-citric acid and non-citrate hydrocarboxylic acid chelators are also believed useful in the present invention such as acetic acid, formic acid, lactic acid, tartaric acid and their salts.

As noted above, the term "chelator" is defined and used herein as a synonym for sequestering agent and is also defined as including molecular encapsulating compounds such as cyclodextrin. Cyclodextrins are cyclic carbohydrate molecules having six, seven, or eight glucose monomers arranged in a donut shaped ring, which are denoted alpha, beta or gamma cyclodextrin, respectively. As used herein, cyclodextrin refers to both unmodified and modified cyclodextrin monomers and polymers. Cyclodextrin molecular encapsulators are commercially available from American Maize-Products of Hammond, Ind. Cyclodextrin are further described in Chapter 11 entitled, "Industrial Applications of Cyclodextrin", by J. Szejtli, page 331-390 of Inclusion Compounds, Vol. III (Academic Press, 1984) which chapter is hereby incorporated by reference.

Preferably the chelator enhances the antimicrobial activity and/or antimicrobial spectrum of the bacteriocin. More preferably the chelator enhances the antimicrobial activity and/or antimicrobial spectrum of the bacteriocin in respect of Gram-negative bacteria and other micro-organisms.

We have found that the provision of a chelator is particularly effective in view of the enhancement of the antimicrobial activity and/or antimicrobial spectrum of the bacteriocin provided. This enhancement is possible irrespective of the manner in which the encapsulated antimicrobial material is delivered or the nature of the shell of the encapsulated antimicrobial material.

Thus in a further aspect the present invention provides an antimicrobial material in an encapsulated form, comprising (a) a core of (i) an antimicrobial material and (ii) a chelator; and (b) a shell of encapsulating material.

In one preferred aspect the encapsulated antimicrobial material further comprises an organic acid, a salt thereof or a mixture thereof. Particularly preferred organic acids are lactic acid and acetic acid. Preferably the organic acids are provided in their salt form such as the sodium salt or potassium salt of the respective acid. Highly preferred organic acid salts are sodium lactate (L-sodium lactate), potassium lactate (L-potassium lactate),

sodium di-acetate and mixtures thereof. Particularly preferred mixtures are mixtures of sodium lactate (L-sodium lactate) and sodium di-acetate; and mixtures of sodium lactate and potassium lactate. Suitable salts of organic acids (and mixtures thereof) are available from Purac, Netherlands under the name PURASAL®.

Each of the preferred aspects described herein are applicable to this aspect of the invention. Particularly preferred aspects include

- wherein the shell of encapsulating material is impermeable to the antimicrobial material.
- the shell is selected to provide sustained release of the antimicrobial material from the encapsulated antimicrobial material.
- the shell is selected to prevent, reduce or inhibit degeneration or inactivation of the antimicrobial material.
- the chelator enhances the antimicrobial activity and/or antimicrobial spectrum of the bacteriocin.
- the chelator enhances the antimicrobial activity and/or antimicrobial spectrum of the bacteriocin in respect of Gram-negative bacteria and other micro-organisms.
- the chelator is selected from EDTA, citric acid, monophosphates, diphosphates, triphosphates and polyphosphates.
- the antimicrobial material is an antibacterial material.
- the antimicrobial material is a bacteriocin.
- the antimicrobial material is at least nisin.

PROCESS

The encapsulated antimicrobial material may be introduced into the foodstuff by (a) injecting the encapsulated antimicrobial material into the foodstuff or (b) tumbling the encapsulated antimicrobial material with the foodstuff.

In one aspect the encapsulated antimicrobial material is introduced into the foodstuff by injecting the encapsulated antimicrobial material into the foodstuff.

In one aspect the encapsulated antimicrobial material is introduced into the foodstuff by tumbling the encapsulated antimicrobial material with the foodstuff.

As noted herein the encapsulated antimicrobial material may be introduced into the foodstuff by means other than injection or tumbling. For example the encapsulated antimicrobial material may be incorporated in a marinade. Marinated meat can be prepared in two ways: 1) a surface treatment (such as, for example but not limited to, adding the marinade to the raw meat followed by gas- or vacuum packing) or 2) forceful incorporation on the marinade/brine by physical means (such as, for example but not limited to, tumbling or injection).

Teachings on the practice of injection into foodstuffs or tumbling of foodstuffs can be found in WO 00/62632.

HIGHLY PREFERRED ASPECTS

Some highly preferred aspects of the present invention are set out below

- a process for introducing an antimicrobial material into a foodstuff comprising (i) providing nisin in an encapsulated form comprising a core of nisin and shell of encapsulating material, and (ii) introducing encapsulated nisin into the foodstuff by (a) injecting the encapsulated nisin into the foodstuff or (b) tumbling the encapsulated nisin with the foodstuff.
- a process for introducing an antimicrobial material into a foodstuff comprising (i) providing nisin in an encapsulated form comprising a core of nisin and shell of encapsulating material, and (ii) introducing encapsulated nisin into the foodstuff by (a) injecting the encapsulated nisin into the foodstuff or (b) tumbling the encapsulated nisin with the foodstuff, wherein the nisin is present in an amount to provide a microbicidal or microbiostatic effect in respect of a micro-organism selected from *Lactobacillus*, *Leuconostoc*, *Carnobacterium*, *Enterococcus*; *Listeria monocytogenes*, *Bacillus*, *Clostridium*; and *Brochothrix thermosphacta*.
- a process for introducing an antimicrobial material into a foodstuff comprising (i) providing nisin in an encapsulated form comprising a core of nisin and shell of encapsulating material, and (ii) introducing encapsulated nisin into the foodstuff by (a) injecting the encapsulated nisin into the foodstuff or (b) tumbling the encapsulated nisin with the foodstuff, wherein the encapsulated nisin has an average particle size of less than 150µm.
- a process for introducing an antimicrobial material into a foodstuff comprising (i) providing nisin in an encapsulated form comprising a core of nisin and shell of

encapsulating material, and (ii) introducing encapsulated nisin into the foodstuff by (a) injecting the encapsulated nisin into the foodstuff or (b) tumbling the encapsulated nisin with the foodstuff, wherein the shell is or comprises a material selected from triglyceride and carrageenan.

- a process for introducing an antimicrobial material into a foodstuff comprising (i) providing nisin in an encapsulated form comprising a core of nisin and shell of encapsulating material, and (ii) introducing encapsulated nisin into the foodstuff by (a) injecting the encapsulated nisin into the foodstuff or (b) tumbling the encapsulated nisin with the foodstuff, wherein the foodstuff is raw meat.
- a process for introducing an antimicrobial material into a foodstuff comprising (i) providing nisin in an encapsulated form comprising a core of nisin and shell of encapsulating material, and (ii) introducing encapsulated nisin into the foodstuff by (a) injecting the encapsulated nisin into the foodstuff or (b) tumbling the encapsulated nisin with the foodstuff, wherein the encapsulated nisin is introduced into the foodstuff in a brine carrier.
- a process for introducing an antimicrobial material into a foodstuff comprising (i) providing nisin in an encapsulated form comprising a core of nisin and shell of encapsulating material, and (ii) introducing encapsulated nisin into the foodstuff by (a) injecting the encapsulated nisin into the foodstuff or (b) tumbling the encapsulated nisin with the foodstuff, wherein the nisin is present in an amount to provide a microbicidal or microbiostatic effect in respect of a micro-organism selected from *Lactobacillus, Leuconostoc, Carnobacterium, Enterococcus; Listeria monocytogenes, Bacillus, Clostridium;* and *Brochothrix thermosphacta*, wherein the encapsulated nisin has an average particle size of less than 150µm.
- a process for introducing an antimicrobial material into a foodstuff comprising (i) providing nisin in an encapsulated form comprising a core of nisin and shell of encapsulating material, and (ii) introducing encapsulated nisin into the foodstuff by (a) injecting the encapsulated nisin into the foodstuff or (b) tumbling the encapsulated nisin with the foodstuff, wherein the nisin is present in an amount to provide a microbicidal or microbiostatic effect in respect of a micro-organism selected from *Lactobacillus, Leuconostoc, Carnobacterium, Enterococcus; Listeria monocytogenes, Bacillus, Clostridium;* and *Brochothrix thermosphacta*, wherein the shell is or comprises a material selected from triglyceride and carrageenan.
- a process for introducing an antimicrobial material into a foodstuff comprising (i) providing nisin in an encapsulated form comprising a core of nisin and shell of

encapsulating material, and (ii) introducing encapsulated nisin into the foodstuff by (a) injecting the encapsulated nisin into the foodstuff or (b) tumbling the encapsulated nisin with the foodstuff, wherein the nisin is present in an amount to provide a microbicidal or microbiostatic effect in respect of a micro-organism selected from *Lactobacillus, Leuconostoc, Carnobacterium, Enterococcus; Listeria monocytogenes, Bacillus, Clostridium;* and *Brochothrix thermosphacta*, wherein the foodstuff is raw meat.

- a process for introducing an antimicrobial material into a foodstuff comprising (i) providing nisin in an encapsulated form comprising a core of nisin and shell of encapsulating material, and (ii) introducing encapsulated nisin into the foodstuff by (a) injecting the encapsulated nisin into the foodstuff or (b) tumbling the encapsulated nisin with the foodstuff, wherein the nisin is present in an amount to provide a microbicidal or microbiostatic effect in respect of a micro-organism selected from *Lactobacillus, Leuconostoc, Carnobacterium, Enterococcus; Listeria monocytogenes, Bacillus, Clostridium;* and *Brochothrix thermosphacta*, wherein the encapsulated nisin is introduced into the foodstuff in a brine carrier.
- a process for introducing an antimicrobial material into a foodstuff comprising (i) providing nisin in an encapsulated form comprising a core of nisin and shell of encapsulating material, and (ii) introducing encapsulated nisin into the foodstuff by (a) injecting the encapsulated nisin into the foodstuff or (b) tumbling the encapsulated nisin with the foodstuff, wherein the encapsulated nisin has an average particle size of less than 150μm, wherein the shell is or comprises a material selected from triglyceride and carrageenan.
- a process for introducing an antimicrobial material into a foodstuff comprising (i) providing nisin in an encapsulated form comprising a core of nisin and shell of encapsulating material, and (ii) introducing encapsulated nisin into the foodstuff by (a) injecting the encapsulated nisin into the foodstuff or (b) tumbling the encapsulated nisin with the foodstuff, wherein the encapsulated nisin has an average particle size of less than 150μm, wherein the foodstuff is raw meat.
- a process for introducing an antimicrobial material into a foodstuff comprising (i) providing nisin in an encapsulated form comprising a core of nisin and shell of encapsulating material, and (ii) introducing encapsulated nisin into the foodstuff by (a) injecting the encapsulated nisin into the foodstuff or (b) tumbling the encapsulated nisin with the foodstuff, wherein the encapsulated nisin has an average particle size of less than 150μm, wherein the encapsulated nisin is introduced into the foodstuff in a brine carrier.

- a process for introducing an antimicrobial material into a foodstuff comprising (i) providing nisin in an encapsulated form comprising a core of nisin and shell of encapsulating material, and (ii) introducing encapsulated nisin into the foodstuff by (a) injecting the encapsulated nisin into the foodstuff or (b) tumbling the encapsulated nisin with the foodstuff, wherein the shell is or comprises a material selected from triglyceride and carrageenan, wherein the foodstuff is raw meat.
- a process for introducing an antimicrobial material into a foodstuff comprising (i) providing nisin in an encapsulated form comprising a core of nisin and shell of encapsulating material, and (ii) introducing encapsulated nisin into the foodstuff by (a) injecting the encapsulated nisin into the foodstuff or (b) tumbling the encapsulated nisin with the foodstuff, wherein the shell is or comprises a material selected from triglyceride and carrageenan, wherein the encapsulated nisin is introduced into the foodstuff in a brine carrier.
- a process for introducing an antimicrobial material into a foodstuff comprising (i) providing nisin in an encapsulated form comprising a core of nisin and shell of encapsulating material, and (ii) introducing encapsulated nisin into the foodstuff by (a) injecting the encapsulated nisin into the foodstuff or (b) tumbling the encapsulated nisin with the foodstuff, wherein the foodstuff is raw meat, wherein the encapsulated nisin is introduced into the foodstuff in a brine carrier.
- a process for introducing an antimicrobial material into a foodstuff comprising (i) providing nisin in an encapsulated form comprising a core of nisin and shell of encapsulating material, and (ii) introducing encapsulated nisin into the foodstuff by (a) injecting the encapsulated nisin into the foodstuff or (b) tumbling the encapsulated nisin with the foodstuff, wherein the nisin is present in an amount to provide a microbicidal or microbiostatic effect in respect of a micro-organism selected from *Lactobacillus, Leuconostoc, Carnobacterium, Enterococcus; Listeria monocytogenes, Bacillus, Clostridium;* and *Brochothrix thermosphacta*, wherein the foodstuff is raw meat, wherein the encapsulated nisin has an average particle size of less than 150μm.
- a process for introducing an antimicrobial material into a foodstuff comprising (i) providing nisin in an encapsulated form comprising a core of nisin and shell of encapsulating material, and (ii) introducing encapsulated nisin into the foodstuff by (a) injecting the encapsulated nisin into the foodstuff or (b) tumbling the encapsulated nisin with the foodstuff, wherein the nisin is present in an amount to provide a microbicidal or microbiostatic effect in respect of a micro-organism

selected from *Lactobacillus, Leuconostoc, Carnobacterium, Enterococcus; Listeria monocytogenes, Bacillus, Clostridium;* and *Brochothrix thermosphacta*, wherein the foodstuff is raw meat, wherein the shell is or comprises a material selected from triglyceride and carrageenan.

- a process for introducing an antimicrobial material into a foodstuff comprising (i) providing nisin in an encapsulated form comprising a core of nisin and shell of encapsulating material, and (ii) introducing encapsulated nisin into the foodstuff by (a) injecting the encapsulated nisin into the foodstuff or (b) tumbling the encapsulated nisin with the foodstuff, wherein the nisin is present in an amount to provide a microbicidal or microbiostatic effect in respect of a micro-organism selected from *Lactobacillus, Leuconostoc, Carnobacterium, Enterococcus; Listeria monocytogenes, Bacillus, Clostridium;* and *Brochothrix thermosphacta*, wherein the foodstuff is raw meat, wherein the encapsulated nisin is introduced into the foodstuff in a brine carrier.
- a process for introducing an antimicrobial material into a foodstuff comprising (i) providing nisin in an encapsulated form comprising a core of nisin and shell of encapsulating material, and (ii) introducing encapsulated nisin into the foodstuff by (a) injecting the encapsulated nisin into the foodstuff or (b) tumbling the encapsulated nisin with the foodstuff, wherein the nisin is present in an amount to provide a microbicidal or microbiostatic effect in respect of a micro-organism selected from *Lactobacillus, Leuconostoc, Carnobacterium, Enterococcus; Listeria monocytogenes, Bacillus, Clostridium;* and *Brochothrix thermosphacta*, wherein the encapsulated nisin is introduced into the foodstuff in a brine carrier, wherein the encapsulated nisin has an average particle size of less than 150μm.
- a process for introducing an antimicrobial material into a foodstuff comprising (i) providing nisin in an encapsulated form comprising a core of nisin and shell of encapsulating material, and (ii) introducing encapsulated nisin into the foodstuff by (a) injecting the encapsulated nisin into the foodstuff or (b) tumbling the encapsulated nisin with the foodstuff, wherein the nisin is present in an amount to provide a microbicidal or microbiostatic effect in respect of a micro-organism selected from *Lactobacillus, Leuconostoc, Carnobacterium, Enterococcus; Listeria monocytogenes, Bacillus, Clostridium;* and *Brochothrix thermosphacta*, wherein the encapsulated nisin is introduced into the foodstuff in a brine carrier, wherein the shell is or comprises a material selected from triglyceride and carrageenan.
- a process for introducing an antimicrobial material into a foodstuff comprising (i) providing nisin in an encapsulated form comprising a core of nisin and shell of

encapsulating material, and (ii) introducing encapsulated nisin into the foodstuff by (a) injecting the encapsulated nisin into the foodstuff or (b) tumbling the encapsulated nisin with the foodstuff, wherein the nisin is present in an amount to provide a microbicidal or microbiostatic effect in respect of a micro-organism selected from *Lactobacillus, Leuconostoc, Carnobacterium, Enterococcus; Listeria monocytogenes, Bacillus, Clostridium;* and *Brochothrix thermosphacta*, wherein the encapsulated nisin is introduced into the foodstuff in a brine carrier, wherein the foodstuff is raw meat.

- a process for introducing an antimicrobial material into a foodstuff comprising (i) providing nisin in an encapsulated form comprising a core of nisin and shell of encapsulating material, and (ii) introducing encapsulated nisin into the foodstuff by (a) injecting the encapsulated nisin into the foodstuff or (b) tumbling the encapsulated nisin with the foodstuff, wherein the nisin is present in an amount to provide a microbicidal or microbiostatic effect in respect of a micro-organism selected from *Lactobacillus, Leuconostoc, Carnobacterium, Enterococcus; Listeria monocytogenes, Bacillus, Clostridium;* and *Brochothrix thermosphacta*, wherein the encapsulated nisin has an average particle size of less than 150µm, wherein the shell is or comprises a material selected from triglyceride and carrageenan, wherein the foodstuff is raw meat, wherein the encapsulated nisin is introduced into the foodstuff in a brine carrier.
- an antimicrobial material in an encapsulated form, comprising a core of nisin and shell of encapsulating material, wherein the shell of encapsulating material is impermeable to nisin.
- an antimicrobial material in an encapsulated form, comprising a core of nisin and shell of encapsulating material, wherein the shell of encapsulating material is impermeable to nisin, wherein the nisin is present in an amount to provide a microbicidal or microbiostatic effect in respect of a micro-organism selected from *Lactobacillus, Leuconostoc, Carnobacterium, Enterococcus; Listeria monocytogenes, Bacillus, Clostridium*; and *Brochothrix thermosphacta.*
- an antimicrobial material in an encapsulated form, comprising a core of nisin and shell of encapsulating material, wherein the shell of encapsulating material is impermeable to nisin, wherein the encapsulated nisin has an average particle size of less than 150µm.
- an antimicrobial material in an encapsulated form, comprising a core of nisin and shell of encapsulating material, wherein the shell of encapsulating material is

impermeable to nisin, wherein the shell is or comprises a material selected from triglyceride and carrageenan.

- an antimicrobial material in an encapsulated form, comprising a core of nisin and shell of encapsulating material, wherein the shell of encapsulating material is impermeable to nisin, wherein the nisin is present in an amount to provide a microbicidal or microbiostatic effect in respect of a micro-organism selected from *Lactobacillus, Leuconostoc, Carnobacterium, Enterococcus; Listeria monocytogenes, Bacillus, Clostridium;* and *Brochothrix thermosphacta*, wherein the encapsulated nisin has an average particle size of less than 150µm.
- an antimicrobial material in an encapsulated form, comprising a core of nisin and shell of encapsulating material, wherein the shell of encapsulating material is impermeable to nisin, wherein the nisin is present in an amount to provide a microbicidal or microbiostatic effect in respect of a micro-organism selected from *Lactobacillus, Leuconostoc, Carnobacterium, Enterococcus; Listeria monocytogenes, Bacillus, Clostridium;* and *Brochothrix thermosphacta*, wherein the shell is or comprises a material selected from triglyceride and carrageenan.
- an antimicrobial material in an encapsulated form, comprising a core of nisin and shell of encapsulating material, wherein the shell of encapsulating material is impermeable to nisin, wherein the encapsulated nisin has an average particle size of less than 150µm, wherein the shell is or comprises a material selected from triglyceride and carrageenan.
- an antimicrobial material in an encapsulated form, comprising a core of nisin and shell of encapsulating material, wherein the shell of encapsulating material is impermeable to nisin, wherein the nisin is present in an amount to provide a microbicidal or microbiostatic effect in respect of a micro-organism selected from *Lactobacillus, Leuconostoc, Carnobacterium, Enterococcus; Listeria monocytogenes, Bacillus, Clostridium;* and *Brochothrix thermosphacta*, wherein the foodstuff is raw meat, wherein the encapsulated nisin has an average particle size of less than 150µm.
- an antimicrobial material in an encapsulated form, comprising a core of nisin and shell of encapsulating material, wherein the shell of encapsulating material is impermeable to nisin, wherein the nisin is present in an amount to provide a microbicidal or microbiostatic effect in respect of a micro-organism selected from *Lactobacillus, Leuconostoc, Carnobacterium, Enterococcus; Listeria monocytogenes, Bacillus, Clostridium;* and *Brochothrix thermosphacta*, wherein the

foodstuff is raw meat, wherein the shell is or comprises a material selected from triglyceride and carrageenan.

The present invention will now be described in further detail in the following examples.

EXAMPLES

Example 1

First, a solution of 15 g k-carrageenan in 1000 mL of phosphate buffer at pH 3,5 is prepared at 85°C. To this is added 300 g of Nisaplin® (Danisco commercial extract of nisin: equivalent to 1 x 10^6 IU/g nisin potency). The resulting mixture is thoroughly mixed. At the same time, a mixture of 1333 g of a vegetable triglyceride (Danisco: GRINSTED ® PS 101, m.p. 58°C) and 73 g of acetylated emulsifier (Danisco: Acetem 50 00) is melted at 85°C in a water bath. The melted fat mixture is kept under homogenisation (Silverson mixer, 8 kRPM) as the aqueous mixture is slowly incorporated. The homogenisation is maintained for 5 minutes after the whole aqueous mixture is added and then a solution of 3 g of polysorbate 80 in 40 mL of water is added under constant mixing. The resulting low-viscosity water-in-oil emulsion is then immediately spray cooled in a Niro spray tower using the following parameters: inlet air temperature: 10°C, outlet air temperature 28 °C, rotating atomization wheel speed: 10 kPRM. A free flowing powder is obtained.

This encapsulated nisin can be used for injection or tumbling of raw meat that is then immediately cooked. Nisin release from the fat shell would occur upon injection and/or cooking. Since the fat-based encapsulated shell material would make the particles float to the surface of the injection brine, either a) a viscosifying agent such as xanthan could be used to stabilise the particles in the brine, or b) to mix the brine before use as an injection material. Mixing of the particles would naturally occur when encapsulated nisin is used in the brine used for tumbling of meat.

The same encapsulated material can be used for sustained release at chilled temperature of encapsulated nisin within marinades used on vacuum-packaged, chilled raw meat.

Example 2

First, a solution of 15 g sodium alginate in 1000 mL of phosphate buffer at pH 3.5 is prepared at 85°C. To this is added 300 g of Nisaplin® (Danisco commercial extract of nisin: equivalent to 1 x 10^6 IU/g nisin potency). The resulting mixture is thoroughly mixed. At the same time, a mixture of 1333 g of a vegetable triglyceride (Danisco: GRINSTED ® PS 101, melting point 58 °C) and 73 g of acetylated emulsifier (Danisco: Acetem 50 00) is melted at 85 °C in a water bath. The melted fat mixture is kept under homogenization (Silverson mixer, 8 kRPM) as the aqueous mixture is slowly incorporated. Following the incorporation of the aqueous mixture, a solution of 7 g of calcium chloride in 70 mL of water is added dropwise. The homogenization is maintained for another 5 minutes and then a solution of 3 g of polysorbate 80 in 40 mL of water is added under constant mixing. The resulting low-viscosity water-in-oil emulsion is then immediately spray cooled in a Niro spray tower using the following parameters: inlet air temperature: 10°C, outlet air temperature 28°C, rotating atomization wheel speed: 10 kPRM. A free flowing powder is obtained.

The use of this encapsulated nisin is as described in Example 1.

Example 3

A solution of 1 g of a bilayer-forming lipid and 100 mg of cholesterol in a suitable organic solvent is evaporated so as to form a thin dry lipid film on the bottom of the container. After thorough drying of the lipid film, 1 L of water containing nisin (as Nisaplin®) at the saturation concentration is added to the container and the mixture is thoroughly mixed or homogenized. The resulting suspension of multilamellar vesicle (MLV) can be further processed by microfluidization to form smaller more homogenous small unilamellar vesicle (SUV). The suspension of liposome-encapsulated nisin can be added directly to the meat by injection/tumbling.

These particles are small enough to pass through injection needles without disintegration of the liposome shell. The liposome-encapsulated nisin would be released on cooking since liposomes break up at 45–50°C because of the transition temperature of bilayer-forming phospholipids/amphiphilic compounds. Lipsome-encapsulated nisin would be slowly released over time, thus making it suitable for sustained release in raw meat marinades.

Liposome-encapsulated nisin can be made by several processes, including microfluidization, extrusion, 'French press', reverse phase evaporation, freeze-thaw cycle, etc. Microfluidization, is the preferred aspect since it is a continuous, high capacity and solvent –free process.

Example 4

Use of a fluidised bed to apply a hydrophobic shell onto the nisin. If the nisin particle size is too fine, the powder can be agglomerated in an suitable equipment using a binder solution (solution of sticky hydrocolloids such as alginate or maltodextrin) in order to obtain a dense powder of particle size between 100-150 micrometres. The appropriate powder is then introduced into the coating chamber of a fluidized-bed microencapsulation unit and fluidized at inlet air flow rate of 5-30 cm/s and temperature up to 50°C to fluidized the particles. A coating material is then sprayed onto the fluidized bed of antimicrobial using a double fluid nozzle and high pressure atomization air.

In one example, a melted mixture of triglyceride and additives is sprayed onto the nisin to form a continuous layer of fat around each individual particle as the melted fat spread and solidifies on the particles. The amount of fat applied can be up to 50%, but no usually no lower than 20% w/w.

In another example, a dispersion of coating material in water or a solution of coating material in ethanol is sprayed onto the fluidized particles and the fluidization air is used to evaporate the solvent or the water, which leaves behind a continuous film of coating material on the antimicrobial particles. Examples of coating material in this case include shellac, zein or any other hydrophobic coating materials.

In order for encapsulated nisin prepared by this method to used for raw meat injection, the particles size must be less than 175 micrometres. In addition, the particle size must be greater than 100 micrometres for the fluidization process to work.

Example 5 - Enhanced antilisterial effect with encapsulated nisin in hot dogs

Encapsulated Nisin is prepared by spray crystallization in accordance with the following procedure. Fully hydrogenated triglyceride (GRINSTED PS101, 100 parts) is melted at

85°C in a water bath. Nisin (64 parts) is pre-heated at 50C and added to the melted triglyceride, kept at 85°C, under vigorous mixing. Mixing is maintained until the mixture becomes smooth and lump-free. The suspension is then pumped to the atomization device of a spray tower in traced pipes maintained at 75-85°C. The atomization device is a "rotating wheel" at 9000 RPM installed at the top of the spray tower. Cooled (3-5°C) air is blown in the spray tower so as to crystallize the atomized droplets of fat/nisin before reaching the walls of the tower. The solidified powder is collected at the bottom of the tower. The powder may be kept at 40°C 2-3 days to allow re-crystallization of the fat phase, if necessary, from the alpha to the beta form. Anti-caking agent, such as calcium stearate or silicon dioxide may be added to a 0,1-1% level to prevent further lumping of the powder.

An inoculation trial with *Listeria monocytogenes* was conducted with hot dogs; this demonstrated the heat-protective benefit of encapsulated nisin.

The formulation of the hot dogs was as follows (raw batch weight basis): 74.1% meat trimmings (lean beef and pork fat), 1.66% NaCl, 1.48% corn syrup solids, 0.74% HMP, 0.37% hydrolysed beef stock, 0.33% sodium tripolyphosphate, 0.37% spice/seasoning mix, 0.037% erythorbate, 0.185 sodium nitrite cure blend, 13.3% added water, 7.4% added water (10%, cook shrink). Nisin was added at 250 and 500 IU/g either as unencapsulated nisin (Nisaplin®, Danisco) or as an encapsulated nisin product. The sausage, which contained 28% fat, then underwent a heating/smoking regime as shown below:

Smokehouse schedule

Time (minutes)	Dry bulb (°F)	Wet bulb (°F)	Relative humidity (%)	Smoke
30	130	96	29	Off
15	140	104	30	On
15	150	112	30	On
15	160	120	31	On
15	170	128	31	On
15	180	134	30	On

The sausages were held to an endpoint internal temperature of 160°F, shower cooled to 95°F then chilled to < 40°F. The sausages were vacuum-packed, six to a bag and surface inoculated with a 5 strain cocktail of *Listeria monocytogenes* and *Listeria innocua* (including environmental isolates).

Nisin levels in the hot dogs were measured the following day and during the 12 week storage at 38 – 40 °F by a bioassay method a horizontal agar diffusion assay method using *Micrococcus luteus* as the indicator organism (Fowler et al. 1975. Society for Applied Bacteriology Technical Series 8: 91-105). This uses an acid/heat extraction step that detects all residual nisin within the samples, even if encapsulated. The hot dogs were also analysed at weekly intervals for counts of *Listeria monocytogenes* and natural contaminant lactic acid bacteria.

The long heat processing resulted in significant nisin loss. Initial nisin levels detected in the hot dogs post-processing were much higher in samples to which encapsulated nisin had been added compared to those with unencapsulated nisin (Nisaplin®, Danisco) (see Figure 1).

The microbiological data from the trial was subjected to multivariate statistical analysis. This concluded that encapsulation achieved a greater initial drop in *Listeria* numbers. The optimum treatment for achieving a shelf life of 84 days was provided by encapsulated nisin (at 500 IU/g), the secondary optimum treatment was Nisaplin® (at 500 IU/g). This further demonstrated the superior efficacy of the encapsulated nisin.

Example 6. Improved nisin levels with encapsulated nisin in a bakery item

Crumpets are high moisture flour-based bakery products that have been implicated in food poisoning outbreaks due to *Bacillus cereus*. The products are stored at ambient temperature and during the 5-day shelf life, surviving heat-resistant spores of *Bacillus cereus* (present in the flour) may germinate and grow, particularly in countries with warm climates. Nisin has been used as a preservative in crumpets to prevent the growth of this pathogen and ensure consumer safety. The cooking process for the crumpets can, however, result in significant nisin loss. This involves heating on a hot plate for 3 – 5 minutes.

The trial described below demonstrated the heat-protective effect of encapsulation,

ensuring a greater percentage of added nisin survived the baking process. Crumpets were prepared by a normal production method, with addition of nisin (as Nisaplin®) or encapsulated nisin (prepared by spray crystallization in accordance with the procedure of Example 5) to the batter before cooking on the hotplate. The crumpets (pH 5.6 – 6.0, water activity 0.8 - 0.9) were then incubated at ambient temperature (21 °C) for 5 days. Nisin levels in the crumpets were measured the following day by a bioassay method (see above).

Test samples

1. Nisaplin® (Danisco). Nisin potency 1 x 10^6 IU/g
2. Encapsulated nisin sample NAP 03228 (Danisco). Nisin potency 5.36 x 10^5

Results of crumpet trial

Test	Sample	Sample addition level	Actual nisin addition level	Initial nisin levels detected by bioassay	Detected nisin levels based on % of nisin addition	Average nisin levels as % of addition
1	Nisaplin® (Unencapsulated nisin)	200 mg/kg	200 IU/g	56 IU/g	28%	22%
2				32.2 IU/g	16%	
3	Encapsulated nisin	200 mg/kg	94 IU/g	49.3 IU/g	52%	51%
4				46.6 IU/g	50%	
5		150 mg/kg	71 IU/g	45.4 IU/g	64%	65%
6				47.2 IU/g	66%	
7		100 mg/kg	47 IU/g	32 IU/g	68%	66%
8				29.6 IU/g	63%	

The average residual nisin levels resulting from encapsulation were 61% compared to average residual nisin levels of 22% for unencapsulated nisin.

Example 7. Improved nisin levels in processed cheese

Encapsulated nisin (prepared by spray crystallization in accordance with the procedure of Example 5) and unencapsulated nisin (Nisaplin®, Danisco) were added to a commercial processed cheese formulation, after which samples of the processed cheese were subjected to a heating step of 10 minutes at core temperatures of 60°C, 80°C and 100°C. After heating, the residual nisin levels were measured in the processed cheese, using heat/acid extraction and the horizontal agar diffusion method.

Sample	Residual nisin detected as a percentage of addition level		
	After 60 °C for 10 minutes	After 80 °C for 10 minutes	After 100 °C for 10 minutes
Unencapsulated nisin (Nisaplin®)	74%	68%	59%
Encapsulated nisin	75%	73%	72%
Encapsulated nisin	90%	84%	75%

The results demonstrate higher nisin levels after the heat treatment for encapsulated nisin samples compared to unencapsulated nisin samples.

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All publications mentioned in the above specification are herein incorporated by reference. Various modifications and variations of the described methods and system of the invention will be apparent to those skilled in the art without departing from the scope and spirit of the invention. Although the invention has been described in connection with specific preferred embodiments, it should be understood that the invention as claimed should not be unduly limited to such specific embodiments. Indeed, various modifications of the described modes for carrying out the invention which are obvious to those skilled in chemistry, biology, food science or related fields are intended to be within the scope of the following claims.

CLAIMS

1. A process for introducing an antimicrobial material into a foodstuff comprising
(i) providing the antimicrobial material in an encapsulated form comprising a core of antimicrobial material and shell of encapsulating material
(ii) introducing encapsulated antimicrobial material into the foodstuff by (a) injecting the encapsulated antimicrobial material into the foodstuff or (b) tumbling the encapsulated antimicrobial material with the foodstuff.

2. A process according to claim 1 wherein the antimicrobial material is an antibacterial material.

3. A process according to claim 2 wherein the antimicrobial material is a bacteriocin.

4. A process according to claim 3 wherein the bacteriocin is selected from lanthionine containing bacteriocins, *Lactococcus*-derived bacteriocins, *Streptococcus*-derived bacteriocins, *Pediococcus*-derived bacteriocins, *Lactobacillus*-derived bacteriocins, *Carnobacterium*-derived bacteriocins, *Leuconostoc*-derived bacteriocins, *Enterococcus*-derived bacteriocins and mixtures thereof.

5. A process according to any one of the preceding claims wherein the antimicrobial material is at least nisin.

6. A process according to any one of the preceding claims wherein the antimicrobial material is present in an amount to provide a microbicidal or microbiostatic effect.

7. A process according to claim 6 wherein the microbicidal or microbiostatic effect is a bactericidal or bacteriostatic effect.

8. A process according to claim 7 wherein the bactericidal or bacteriostatic effect is in respect of Gram-positive bacteria.

9. A process according to claim 7 wherein the bactericidal or bacteriostatic effect is in respect of an organism selected from species of *Bacillus*, species of *Clostridium*, *Listeria monocytogenes*, lactic acid bacteria, *Leuconostoc*, *Carnobacterium*, *Enterococcus*; *Brochothrix thermosphacta* and *Lactobacillus* species.

10. A process according to claim 7 wherein the bactericidal or bacteriostatic effect is in respect of *Listeria monocytogenes*.

11. A process according to any one of the preceding claims wherein the shell of the encapsulated antimicrobial material is capable of withstanding injection.

12. A process according to any one of the preceding claims wherein the shell of the encapsulated antimicrobial material is capable of withstanding a pressure of greater than 1.5 bar.

13. A process according to any one of the preceding claims wherein the encapsulated antimicrobial material is a particulate form.

14. A process according to any one of the preceding claims wherein the encapsulated antimicrobial material has an average particle size of less than 150μm.

15. A process according to any one of the preceding claims wherein the shell is selected to provide sustained release of the antimicrobial material from the encapsulated antimicrobial material.

16. A process according to any one of the preceding claims wherein the shell is selected to prevent, reduce or inhibit degeneration or inactivation of the antimicrobial material.

17. A process according to claim 16 wherein degeneration is by one or more factors selected from heat degradation, pH induced degradation, protease degradation and glutathione adduct formation.

18. A process according to any one of the preceding claims wherein the shell is or comprises a material selected from fats, emulsifiers, waxes (animal, vegetable, mineral or synthetic), liposome-forming lipids, hydrocolloids, natural or synthetic polymers and mixtures thereof.

19. A process according to claim 18 wherein the lipid is a glycerophospholipid or and sterol.

20. A process according to claim 18 or 19 wherein the fat is a triglyceride.

21. A process according to claim 20 wherein the triglyceride is a vegetable triglyceride.

22. A process according to any one of claims 18 to 21 wherein the emulsifier is selected from polysorbates, monoglycerides, diglycerides, acetic acid esters of mono-diglycerides, tartaric acid esters of mono-diglycerides and citric acid esters of mono-diglycerides.

23. A process according to any one of claims 18 to 22 wherein the hydrocolloid is cross linked.

24. A process according to claim 23 wherein the hydrocolloid is carrageenan.

25. A process according to any one of the preceding claims wherein the encapsulated antimicrobial material is prepared by or is obtainable by a process selected from spray cooling, and fluidised bed coating.

26. A process according to any one of the preceding claims wherein the foodstuff is selected from raw meat, cooked meat, raw poultry products, cooked poultry products, raw seafood products, and cooked seafood products.

27. A process according to claim 26 wherein the foodstuff is raw meat.

28. A process according to claim 26 wherein the foodstuff is selected from raw meat products, cooked meat products, raw seafood products, cooked seafood products, raw poultry products and cooked poultry products.

29. A process according to claim 26 wherein the foodstuff is a raw or cooked poultry product.

30. A process according to claim 26 or 27 wherein the foodstuff comprises whole meat muscle.

31. A process according to any one of the preceding claims wherein the encapsulated antimicrobial material is introduced into the foodstuff in a carrier.

32. A process according to claim 31 wherein the carrier is or comprises brine.

33. A process according to claim 31 or 32 wherein the carrier and the encapsulated antimicrobial material have substantially the same density.

34. A process according to claim 33 wherein the encapsulated antimicrobial material is modified to have substantially the same density as the carrier.

35. A process according to claim 34 wherein the encapsulated antimicrobial material is modified by contacting the encapsulated antimicrobial material with oil.

36. A process according to claim 35 wherein the oil is brominated oil.

37. A process according to claim 33 wherein the carrier is modified to have substantially the same density as the encapsulated antimicrobial material.

38. A process according to claim 37 wherein the carrier comprises xanthum gum.

39. A process according to any one of claims 33 to 35 wherein the carrier comprises an emulsifier.

40. A process according to any one of the preceding claims wherein the encapsulated antimicrobial material further comprises a chelator.

41. A process according to claim 40 wherein the chelator is selected from EDTA, citric acid, monophosphates, diphosphates, triphosphates and polyphosphates.

42. A process according to claim 40 or 41 wherein the chelator enhances the antimicrobial activity and/or antimicrobial spectrum of the antimicrobial material.

43. A process according to claim 40, 41 or 42 wherein the chelator enhances the antimicrobial activity and/or antimicrobial spectrum of the antimicrobial material in respect of Gram-negative bacteria.

44. A process according to any one of the preceding claims wherein the encapsulated antimicrobial material is introduced into the foodstuff by injecting the encapsulated antimicrobial material into the foodstuff.

45. A process according to any one of claims 1 to 43 wherein the encapsulated antimicrobial material is introduced into the foodstuff by tumbling the encapsulated antimicrobial material with the foodstuff.

46. A process according to claim 1 wherein
(i) the antimicrobial material is at least nisin;
(ii) the antimicrobial material is present in an amount to provide a microbicidal or microbiostatic effect in respect of *Listeria monocytogenes*;
(iii) the shell is selected to prevent, reduce or inhibit degeneration or inactivation of the antimicrobial material by one or more factors selected from heat degradation, pH induced degradation, protease degradation and glutathione adduct formation; and
(iv) the foodstuff is selected from raw meat products, cooked meat products, raw seafood products, cooked seafood products, raw poultry products and cooked poultry products.

47. An antimicrobial material in an encapsulated form, comprising (i) a core comprising an antimicrobial material and (ii) a shell of encapsulating material, wherein the shell of encapsulating material is impermeable to the antimicrobial material.

48. An antimicrobial material according to claim 47 wherein the antimicrobial material is an antibacterial material.

49. An antimicrobial material according to claim 47 or 48 wherein the antimicrobial material is a bacteriocin.

50. An antimicrobial material according to claim 49 wherein the antimicrobial material is at least nisin.

51. An antimicrobial material according to any one of claims 47 to 50 wherein the shell is selected to provide sustained release of the antimicrobial material from the encapsulated antimicrobial material.

52. An antimicrobial material according to any one of claims 47 to 51 wherein the shell is selected to prevent, reduce or inhibit degeneration or inactivation of the antimicrobial material.

53. An antimicrobial material according to any one of claims 47 to 52 wherein the shell is selected to release the antimicrobial material from the encapsulated antimicrobial material under predetermined conditions.

54. An antimicrobial material according to any one of claims 47 to 53 wherein the shell is selected to release the antimicrobial material from the encapsulated antimicrobial material on contact with a foodstuff.

55. An antimicrobial material according to claim 49 wherein the foodstuff is a marinade.

56. A foodstuff prepared by a process as defined in any one of claims 1 to 46.

57. A foodstuff obtainable by a process as defined in any one of claims 1 to 46.

58. A process as substantially hereinbefore described with reference to any one of the Examples.

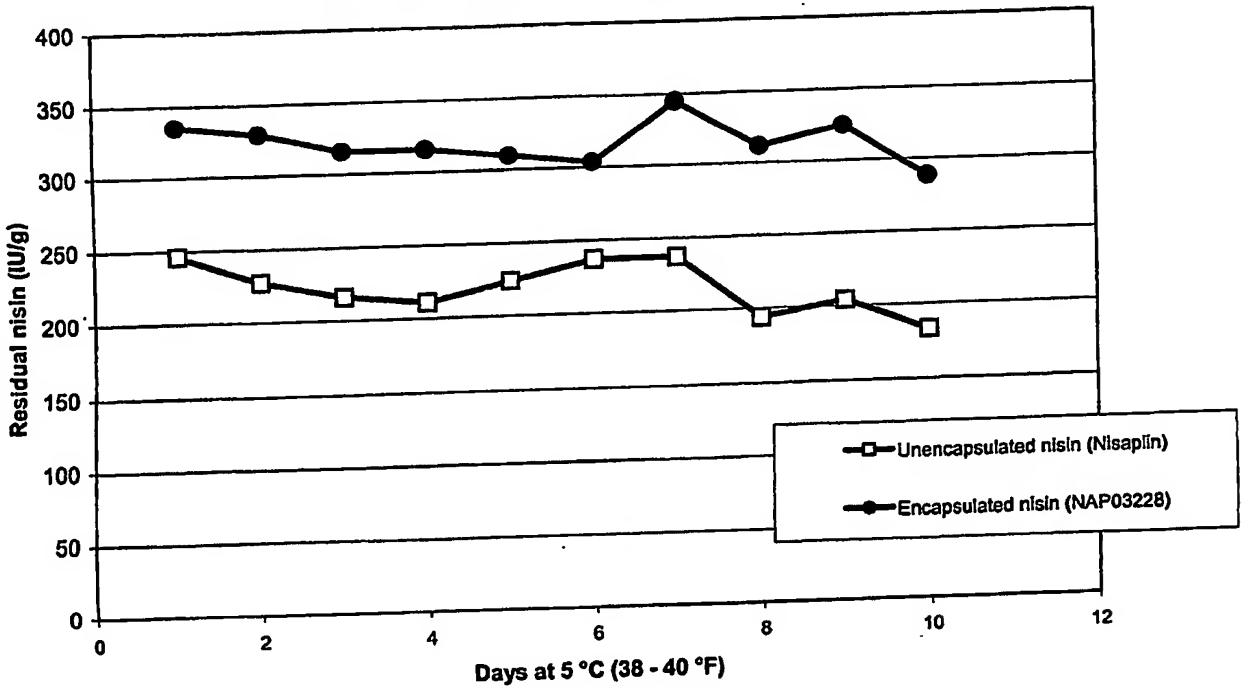
59. A foodstuff as substantially hereinbefore described with reference to any one of the Examples.

ABSTRACT

PROCESS

The present invention provides a process for introducing an antimicrobial material into a foodstuff comprising (i) providing the antimicrobial material in an encapsulated form comprising a core of antimicrobial material and shell of encapsulating material, and (ii) introducing encapsulated antimicrobial material into the foodstuff by (a) injecting the encapsulated antimicrobial material into the foodstuff or (b) tumbling the encapsulated antimicrobial material with the foodstuff.

Figure 1. Nisin levels in the hot dogs after processing.
Equivalent levels of nisin had been added:
more survived the heat processing if encapsulated



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